بسم الله الرحمن الرحيم

Thermal Death of Bacteria Associated with Mutton, Beef and Camel Meats in Khartoum state

By

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Dedication

To my dear father

To my dear mother

To my brothers and sisters

To my friends

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First and foremost, I would like to thank my Merciful Allah for giving me strength and health to do this work.

I would like to deeply thank my supervisor Prof. Suleiman Mohamed ElSanousi for his continuous encouragement and patience throughout the period of this work, without his suggestions, care, consideration and helpful comments, this thesis would likely not have matured.

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Abstract

This study was carried out to identify aerobic bacteria that contaminating the beef, mutton and camel meats and to evaluate their tolerance to different temperatures for different times.

Fresh beef and mutton samples were randomly collected from Khartoum North and Omdurman market, while camel meat samples were collected from slaughter house, total number of samples were 53(23beef,23 mutton,7camel).

Samples were cultured on blood agar; isolates after being purified were identified according to their morphological and biochemical properties to the species level according to Barrow and Feltham (2003).

The isolated Gram-positive bacteria were: *Staph caseolyticus, Micrococcus varians(22.6%),Kurthia gibsonii(24.5%),Bacillus mycoides(16.9%),Stomatococcus mucilaginosus(16.9%) and Enterococcus mundtii.*

The isolated Gram-negative bacteria were: *Escherichia coli (16.9%), Vibrio parahaemolyticus,Aeromonas hydrophila ,Aeromonas salmonocida(1.9%) Enterobacter gergoviae ,Citrobacter freundii, Mannheimia haemlytica, Vibrio furnissii, Vibrio cincinnatiensis,Vibrio metschenikovii andProteus penneri* .

Camel meat was found least contaminated and mutton was most contaminated meat.

The isolated organisms were subjected to different temperatures for different times viz: 70˚C for30 and 60 min, 80˚C for15 and 30 min, 90˚C for 5, 10 and15 min, 100˚C for 5 and 10 min.

The thermal death time at specific temperatures for all isolates was determined. The most heat resistant was *B.mycoides* which survived all set temperatures including 100˚C for 10 minutes .

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ملخص الاطروحة

فى هذه الدراسة جمعت عينات لحوم ضان و عجالى عشوائيا من ا سواق التجزئة ببحرى و امدرمان، بينما جمعت عينات من لحم الابل من مسلخ كرري بامدرمان.

بلغ عدد العينات التي جمعت 53 (23 من لحم الضان ،23من لحم العجالي و 7من لحم الابل)

اجريت هذه الدراسة للتعرف على انواع البكتيريا الهوائية الملوثة للحوم الضان والعجالى والابل واختبار مدى تحملها لدرجات حرارة مختلفة لفترات زمنية مختلفة لتحديد درجة الحرارة التى تعمل على قتلها .

العدد الكلى للعزلات البكتيرية بلغ 70 عزلة تم تعريف الباكتيريا المعزولةوفقا لصفاتها المزرعية والكيموحياتيه استنادا على الطرق الباكتيريولوجية فى دليل(Barrow) and Feltham2003.

الباكتيريا المعزوله الموجبة لصبغة جرام شملت:

Staph caseolyticus, Bacillus mycoides, (16.9%), Micrococcus varians(22.6%), , Kurthia gibsonii(24.5%), Enterococcus mundtii, Stomatococcus mucilaginosus.(16.9%).

بينما شملت الباكتيريا المعزولة السالبة لصبغة جرام :

Escherichia coli(16.9%), , Proteus penneri,Vibrio furnissii, V.metschenikovii, V.cincinnatiensi, Aeromonas salmonocida(1.9%) , A.hydrophila, Citrobacter frundii, Mannheimia haemolytica.

هذه الميكروبات اخضعت لدرجات حراره وفترات زمنية مختلفة (70°م لمدة 30و60 دقيقة،80 °م لمدة 15و30 دقيقة ، 90°م لمدة5 ، 10و15 دقيقة و 100°م دقيقة. لمدة 5و10).

لم تستطع اي من انواع الباكتيريا النمو في اجار الدم في درجات الحرارة والمدة الزمنية المعينة المزكورة انفا، ماعدا *B.mycoides* .

CONTENTS

CHAPTER ONE
INTRODUCTION

Meat is defined as the flesh of animals used as food (Lawrie, 1974). Legally and internationally, the Codex Alimentarius, and the Food and Drug Administration (FDA) in U.S.A, define word meat as: the Parts of the hygienically slaughtered

animal intended for human consumption. It is known that all sound healthy fresh foods generally and especially meat, are found by god in nature in an absolutely sterile condition (Frazier, 1990). Contamination of food starts from the beginning of food harvesting. Meat starts to contaminate at slaughter plants, from environment, labores, utensils, knives, hands and clothes. Secondly, meat is contaminated at transport to retail shops and finally during display in these shops.

Beef is meat from full-grown cattle about 2 years old. A live steer weighs about 1,000 pounds and yields about 450 pounds of edible meat. There are at least 50 breeds of beef cattle, but fewer than 10 make up most cattle produced. Sheep mutton is meat from a sheep over two years old, and has a less tender flesh.

In general, the darker the color, the older the animal. Baby lamb meat will be pale pink, while regular lamb is pinkish-red. Camel meat has the approval of the National Heart Foundation. It is high in protein and low in fat.

Most of the microbial load on the carcass is derived from the skin, hide or fleece during skinning. Bacterial contamination includes the normal skin flora as well as organisms from soil and faeces which are on

the skin. Organisms Include *yeasts, Bacilli, Micrococcus, Staphylococci, Corynebacteria, Moraxella, Acinetobacter, Flavobacteria, Enterobacteriaceae, E coli, Salmonella, Listeria spp, (*ICMSF, 1998*).*

The predominant contaminat is the mesophilic bacteria, the percentage of psychrophilic bacteria varies with season and geographic location. Sometimes animals are washed before slaughter to remove loose dirt, however, it is not clear whether pre-slaughter washing has any significant effect on microbial contamination of carcass (Roberts, 1980 and Gill,1990).

Knives and operators are first used to separate the skin from the under lying hock; thus the skin becomes heavily contaminated, as do their knives, steels and clothes. Salmonellae can often be found on the hands and equipment of these workers (Smeltzer *et al.*, 1980 and Stolle, 1981).

Bacterial numbers are highest on region of the carcass where the initial manual removal of the skin takes place and lowest where the skin is mechanically pulled away (Kelly *et al.*, 1980).

The internal tissues of a healthy animal are known to be sterile and free of microorganisms (Jay, 2005, Thoronton, 1976 and Gracy, 1986).

Contamination at and after slaughter seems to be the only source of microorganisms, their primary sources and roots are:

The stick knives, animal hides or skin, gastero intestinal tract, hands of handlers, containers, utensils and storage environment.

Meat has a high water and protein content, low in carbohydrates, and contains a number of low- molecular weight soluble constituents.

Meat is a highly nutritious substrate with water suitable for the growth of most microorganisms.

Microorganisms can grow in a wide range of temperatures. Since they depend on

water as a solvent for nutrients, frozen water or boiling water inhibits their growth.

Bacteria need certain conditions to grow in food such as warmth, moisture, nutrients and a special pH. Most of the pathogenic bacteria in food multiply rapidly between 10 to 60 degrees Celsius. That is the reason why cold food always should be stored lower than seven degrees in the fridge. This slows down the growth of bacteria. Cooked food should at least be heated to 70 degrees Celsius in the centre for two minutes. Cooking at temperatures between 70° and 100°C kills most bacteria but some spores can survive and can give rise to growth of bacteria if food is later stored below 60°C. It is better to cool cooked foods as quickly as possible, then refrigerate. Reheat cooked foods thoroughly to kill any bacteria, which may have developed during storage. It is important to know about meat and the bacteria inside meat for more than one reason, so we can know when and how to cook our food to be safe and healthy.

Temperature helps control bacterial life and death. Temperatures that are too hot or too cold will kill some or all bacteria.

The temperature of food plays a big role in assuring that certain products are well enough cooked to kill harmful organisms like bacteria. Similarly, many foods, including cooked food, become breeding grounds for other harmful organisms if unrefrigerated too long or even if left in a refrigerated environment for too long a time.

Certainly in the cooking area it is quite straightforward to monitor the internal temperature of meats and other foods to assure that the proper minimum temperature has been attained before it is considered safe.

OBJECTIVES

This work was undertaken to study:

1. Bacteria contaminating beef, mutton and camel meats.
2. The thermal death of such isolated bacteria.

CHAPTER TWO

LITERATURE REVIEW

2.1GENERAL OVERVIEWS

The food poisoning bacteria *Escherichia coli*, *Shigella spp*, *Clostridium perfringens* and *Staphylococcus aureus* were isolated from beef chops which were mixed with spices, onion and garlic (Fatima, 1990).

Hussein (1987) recorded that the aerobic bacterial genera isolated from fresh and refrigerated beef samples were *Bacillus*, *Staphylococcus epidermidis*, *Diphtheroid*, *Micrococcus*, *Streptococcus faecalis*, *Lactobacillus, Escherichia coli*, *Citrobacter freundii*, *and Proteus morgani*, *Alcaligens*, *Aeromonas* and *Pseudomonas*. No salmonellae were detected.

Salih (1971) found fresh meat samples were heavily contaminated with spoilage bacteria of the genera *Micrococcus*, *Streptococcus*, *Bacillus*, *Clostridium*, *and Pseudomonas*.

Sariy Eldin and Ibrahim (1977) investigated the bacterial load of freshly dressed beef carcasses at Omdurman central Abattoir. They found that 52% of the samples had an aerobic proteolytic bacteria.

Imwidthaya, Komopis, Anukarah and Charasert (1987) found that fresh meat samples were contaminated mainly by Gram-negative bacteria.

Borah, Patgiri and Boro (1992) reported that large numbers of pathogenic bacteria were among the organisms isolated from market pork

which included *Klebsiella pneumoniae*, *Enterobacter agglomerans*, *Enterobacter cloacae*, *Escherichia coli*, *Hafnia alvei*, *Citrobacter diversus*, *Citrobacter freundii*, *Serratia marcescens*, *Proteus spp*, *Pseudomonas aeruginosa*, *Alcaligenes faecalis*, *Staphylococcus aureus*, coagulase- negative *Staphylococcus*, Bacilli, Micrococci and Enterococci. Coliforms were the most frequent of the isolates from the meat laboratory and from the market.

EL Gohary, Samaha and Mousa (1993) isolated *Yersinia enterocolitica* and *Yersinia intermedia* from sausage and luncheon.

Brahmbhalt and Anjaria (1993) reported that a total of 66 bacterial isolates were obtained from 54 aseptically collected samples of raw meat obtained from shops. The isolates consisted of *Staphylococcus epidermidis*, *S.aureus*, *E.coli*, *Micrococcus luteus*, *Citrobacter freundii*, *Bacillus cereus*, *Streptococcus faecalis*, *Enterobacter aerogenes*, *Proteus mirabilis*, *Bacillus subtilis*, *Aeromonas liquifaciens*, *P. vulgaris*, *Klebsiella pneumoniae*, *and Pseudomonas aeruginosa*. Nine of eleven *S.aureus* were coagulase–positive and therefore potentially pathogenic.

Bersain,Cantoni and D'Aubert (1991) identified the spoilage bacteria *Pseudomonas putida* and *Actinobacter wolfi* in dark meat of elevated pH.

Lawrie (1979) reported that the superficially recognizable signs of aerobic bacteria were slime formation, discolouration by destruction of meat pigments or growth of colonies of colored organisms, production of off-odours, taints and fat decomposition.

The most common indicators of spoilage were odour, slime, bone taint and discoloration (ICMSF, 1980). Among the early signs of

spoilage of ground beef was the development of off-odour followed by the presence of bacterial slime (Ayres, 1960).

Gill (1976) indicated that slime, off-odor and ammonia could be detected as bacterial population on the surface of meat.

Frazier (1967) reported that any contaminating bacteria on the knife soon would be found on meat in various parts of the carcass carried by blood.

Lawrie (1979) showed in his book that if an infected knife was used or organisms were inadvertently introduced from the skin where the main blood vessels were being severed, bleeding could lead to bacteraemia and to the infection of the tissue.

Gracy (1980) pointed out that bacterial contamination of meat occurred by different sources. First of all, an early invasion of blood vessels by bacteria from the intestines of awakened or ill animals just prior to slaughter.

Gracy (1980) also pointed out that one serious source of contamination from digestive material was the regurgitation which occurred when cattle were stunned and bled. The digest caused severe contamination of the neck, throat and tongue. Work in Australia had shown that the level of contamination of beef carcasses with intestinal organisms was often higher on the inner aspect of the neck than in other

locations of the carcass.

Visser and Bijker (1988) reported that the hair coats of 20 calves were heavily contaminated with bacteria of faecal origin. Including drug resistant bacteria of the family Enterobacteriaceae, Pseudomonas and type D streptococci, these bacteria presented a meat hygiene problem.

FAO/WHO (1962) defined meat hygiene to start from the animal being on the farm through its journey till it reached the consumer as fresh, whole some, sound and safe meat.

Gracy (1980) related bacteria associated with meat to the bacteriology of soil on which the animals were kept prior to slaughtering. The bacteria were transferred to the hide and to exposed meat. He also stated that contamination of meat readily occurred during handling.

Al Aboudi,Hammed and Hassan (1989) reported that seven different bacteria viz: *E.coli, Klebsiella, Proteus, Pseudomonas, Salmonella, Shigella* and *Staphylococcus* were isolated from raw and cooked meat as well as from hands of food handlers and utensils. Five salmonella serotypes were identified from cooked meat and employers hands and the most predominant serotype was *S.tennessee*. Serotyping of meat isolates of *E.coli* showed the presence of many different potential pathogenic strains.

Fathi, Rashwan and El-Syiad (1992) reported that recovery of the coliforms and *E.coli* from some selected meat products was determined. The presence of coliforms in all samples was attributed to contamination of raw meat used for manufacture of such products. In addition, meat products might be also contaminated with *E.coli* from food handlers, food utensils, air, soil and water under incomplete hygienic circumstances during manufacturing, packing and marketing of these products.

The viability of heat-stressed cells of *Escherichia coli* was influenced by the temperature of incubation prior and subsequent to heat treatment. The effect of pre-incubation temperature on the viability of

heated cells was almost constant regardless of heating temperature in the range 48–54°C. The involvement of the change in fluidity of the membrane was suggested as a cause of the effects of pre-incubation and post-incubation. These phenomena were observed with other Gram negative and positive bacteria, and yeasts (Katsuin,Tsuchido andTakano. 1982).

Nashwa (2004) determined the thermal death time of the isolated organisms by the boiling test. The thermal death time ranged between two minutes and more than ten minutes.

When normal milk of good quality is efficiently pasteurized, the type remaining depends on the number of heat resisting organisms that were in the milk before pasteurization and the temperature to which it was heated. Two principal types of bacteria that survive pasteurilization at 145˚F are the thermoduric and the thermophilic.

The thermoduric organisms differ from the thermophilic bacteria in having a lower optimum growth temperature.

The thermophilic have an optimum growth temperature near that used in pasteurization.

The thermoduric organisms are two types:

Spore-formers and non spore-formers, which have a greater resistance to temperature that desteroy the ordinary bacteria (Henderson, 1971). Nashwa (2004) reported the processing of the *Staphylococci*, *Micrococci*, *Streptococci*, *Stomatococci*, *Bacillus* spp, *Corynebacterium*, *Listeria, Rothia* spp, *Pasteurella* spp, *Moraxella* spp, *Vibrio* spp, *Flavobacterium* spp, *Shigella* spp, *Achromobacter* spp, *Acinetbacter* spp,

Branhamella spp,to be found in pasteurized milk and boiling milk to kill this bacteria is essential.

The bacterial attachment to the meat surfaces might depend not only on the physiological state of bacteria,but also an ambient temperature (Fletcher, 1977).

Nortje and Naude (1981) reported that there was a significant interaction between the microbiology variation and carcass characteristics including weight and fat content.

The pH of meat is naturally about 7.0 which is nearly optimal for the growth of many pathogenic and spoilage bacteria (Silliker *et al.*, 1980).

Much of the contamination was originally of faecal origin, but it might include the normal microbes of the skin (*Staphylococci*, *Micrococci*, *Pseudomonas*)as well as organisms from the soil and water (Nortije *et al.*, 1990).

With the exception of external surface, the gastro-intestinal and respiratory tract, and the tissue of normal healthy animals contain few organisms (Silliker *et al.*, 1980).

The prevailing temperature of soil influences the proportion of the psychrotrophic organisms contamination from this source. Tropical soils contain fewer psychr-

otrophic bacteria than soils from temperate zones, and the organisms on the skin of cattle and on meat follow similar trend (Empey and Scott, 1939, Larkin, 1970).

Some microorganisms of human origin reached the meat through the contamin-ation of hands and clothing (Wagner, 2000).

The more distant the market from the slaughter house, the high level of the bacteria contamination of the meat (Silliker *et al*, 1980).

When the meat was transported uncovered exposed to the air current, it would be harboring many types of bacteria (Wagner, 2000).

Water used for washing may be a potential source of contamination of meats with *Enterococci* (Kenner and Kabler, 1960. Salih, 1971).

Organisms of the genus *Micrococcus* are widely distributed and are found as a part of the flora in many food products (Riemann, 1969).

Salih (1971) isolated *Micrococci* from samples of sheep and bovine offals collected from Omdurman central slaughter house and retail meat markets.

Escherichia coli is an indicator bacteria of faecal contamination, enteropathogenic *E.coli* is a significant cause of diarrhea in developing countries and localities of poor sanitation (Wagner, 2000).

Feces and untreated water are the most likely sources for contamination of food, so detection of *E.coli* in meat is considered of public health importance (Wagner, 2000). Dolman (1967) reported that members of this genus are common inhabitants of the intestinal tract of man, animals and decaying materials. They may be isolated from spoiled meat, and also prevalent in fresh meats which are subjected to much handling.

Aeromonas spp can be found in water and soil (Kaper *et al*.,1981). Most are non- pathogenic or of low pathogenicity.

Aeromonas hydrophila, however has been reported as causing septicemia, cellulitis, meningitis and acute diarrheal disease resembling cholera (Monica, 2000). *Aeromonas spp* are greatly affected by elevated

temperatures above 45˚C (Hazen, 1979; Hazen and Fliermans, 1979; Rippey and Cabelli, 1979;Silliker *et al*., 1980).

Aeromonas spp have been found to be common contaminants in foods of animal origin (Myers *etal*., 1982; Egan, 1984; Gray, 1984, Palumbo,Morgan and Buchanan 1985; Okrend,Rose and Bennett, 1987).

In the Sudan, Sanousi *et al*., (1986), isolated *A.hydrophylia* from meat products.

Quality control of meat depends not only on the resolution of all those problems associated with growth nutrition, conformation, slaughtering, and cooling but also on meat hygiene.The objective of meat hygiene is to provide wholesome meat and products which do not constitute a danger to public health (Herschdoerfer,1986).

At the time of slaughter the meat portion of a normal healthy animal may be regarded as essentially free from bacteria (Gill, 1982).

Gram –negative psychrotrophs were recovered from the hides, from structural and work surfaces within the slaughterhouse and from carcasses and meat at all stages of processing (Newton,Harrison and Wauters,1978).

The first major source of contamination affecting meat was from the skin or hide of particular animal being dressed and others in close proximity (Stringer,Bilskie and Naumann, 1969, Grau, 1974, Nottingham *et al*., 1974, and Silliker *et al*., 1980).

The temperature range for growth and toxin production by *S.aureus* was about 4-46˚C, it has been reported that toxin production was best at 40˚ C (Dolman, 1967 Frazier and Westhoff, 1978).

A million of Staphylococci permillitre or gram of perishable foods would be inactivated by 66˚ C maintained for at least 12 minutes or by 60˚ C for 78-83 minutes. (Frazier and Westhoff, 1978).

Moist heat readily kills viruses, bacteria, and fungi. Exposure to boiling water for ten minutes is sufficient to destroy vegetative cells and eukaryotic spores. Unfortunately the temperature of boiling water (100˚C or 212 ˚F) is not high enough to destroy bacterial endospores, which may survive hours of boiling. Therefore, boiling can be used for disinfection of drinking water and objects not harmed by water. But boiling does not sterilize.

Because heat is so useful in controlling microorganisms, it is essential to have precise measure of the heat–killing efficiency.

Thermal death point (TDP)

The lowest temperature at which a microbial suspension is killed in 10 minutes.

Thermal death time (TDT)

The shortest time needed to kill all organisms in a microbial suspension at specific temperature and under defined condition.

TEMPERATURE:

Temperature is the most important factor influencing the growth, it affects the enzyme – catalyzed reaction.

The temperature inhances the growth rate because the velocity of an enzyme – catalyzed reaction increases.

Because the rate of each reaction increases the metabolism and this is more active at higher temperatures, as the microorganism grows faster. Beyond a certain point further increases actually slow growth, and

sufficiently high temperatures are lethal (Prescott,Harleyand Klein, 1999).

High temperatures damage microorganisms by denaturing enzymes, transport carriers, and other proteins. Microbial membranes are also disrupted by heat.

Controlling microbial populations in foods by means of high temperatures can significantly limit the disease transmission and spoilage. Pasteurization uses high temperatures to eliminate disease – causing organisms and reduce microbial populations. Sterilization relies on high temperatures to eliminate all living organisms. Pasteurization involves heating food to a temperature that kills disease –causing microorganisms such as *Mycobacterium tuberculosis* and substantially reduces the levels of spoilage organisms. In the processing of milk, beers, and fruit juices by conventional low- temperature holding (LTH) pasteurization, the liquid is maintained at 62.8˚C for 30 minutes. Products can also be held at 71˚C for 15 seconds, while in the high-temperature, short- time (HTST) process; milk can be treated at 141˚C for 2seconds the ultra – high – temperature (UHT) processing results in improved flavor and extended product shelf life (Prescott *et al*., 1999).

Excess heat energy can cause proteins to become denatured, meaning that they lose their normal three-dimensional shape. Effective temperature for the reduction of microbes is measured as the thermal death point (TDP) for each organism, which is the temperature at which all growth stops. Thermal death time (TDT) is the amount of time it takes to kill all of the microbes in a sample, and the decimal reduction factor (DRF) is the amount of time at a specific heat necessary to reduce

the population of microbes in a sample to tenfold. The most common methods of applying excess heat energy are flaming and incineration, which destroy all life completely. Flaming of inoculating loops and needles, as well as the tops of glass culture tubes and flasks insures that no contaminating microbes can infect sterile media. Applying dry heat by forcing hot air onto the surface of an object can be used in a similar fashion, though many spore formers are capable of withstanding this. The application of moist heat, such as boiling, steaming, and pasteurization (application of high heat to a solution for a short period of time), is also commonly used. These methods work well for most microbes, but are incapable of killing organisms which are thermoduric (capable of withstanding elevated temperatures), or are spore formers. For example, the spores of *Clostridium botulinum*, the bacterium which causes botulism, can be boiled for up to five hours and still remains viable. The most effective application of moist heat is through the use of a device called an autoclave. The autoclave works on the principle of saturated steam. The inner chamber is raised to an air pressure of 15 lb/inch, and then steam at a temperature of 121^{o} C is injected. The steam strikes the surface of the object to be sterilized and condenses into water as its excess heat energy is released. This condensation creates a partial vacuum which draws more steam to the object. Saturated steam is extremely effective as a sterilizing agent, at least 1500 times more effective than the application of dry heat. Autoclaves are usually operated in cycles between 15 and 90 minutes, and can be used to sterilize glassware, surgical implements, soil, water, and microbiological media such as broths and agars. They cannot, however, be used to sterilize

hydrophilic powders which would clump, or hydrophobic oils since microbes suspended in oils would only be subjected to dry heat. Also, while contaminated bandages can be placed in an autoclave, the toxins or exoenzymes left behind by killed microorganisms such as *Clostridium perfringens* (the agent of gas gangrene) may still be capable of causing host cell damage, so these should be rinsed thoroughly with sterile water prior to reuse (Jay *et al*., 2005).

All of the above physical means of control can be checked for effectiveness utilizing various bacteria as quality control agents. Devices which emit ionizing radiation can be tested with *Micrococcus radiouridans*, U.V. devices with *Bacillus pumilis*, and heat disinfecting and sterilizing units such as hot-air ovens, pressure cookers, and autoclaves with *Bacillus stearothermophilus*. These organisms are generally supplied to laboratories live or in ampules or tape strips, which can be placed in the control device. After a normal operating cycle, the organisms are incubated in microbiological media. If growth occurs, the device is not operating properly and should be repaired. Quality control checks and maintenance are vital to the effective microbiological laboratory or health-care facility, and should be performed on a regular basis to prevent contamination and the spread of disease (Jay,Martin,Loessner and David, 2005).

Effects of heat on bacteria making up part of the micro flora of the sea-fish *Trachurus trachurus* were studied (Vaz-Pires, 1996). From 80 bacterial isolates which were subjected to primary tests for the identification, four were selected. The bacteria were subjected to a heat stress of 55°C for 20 s both in pure and mixed suspensions; the D-values

and post-stress recovery in nutrient broth were measured. Using differential plating on selective agents to identify sites of injury and recovery times. The growth parameters, dissolved oxygen, redox potential and pH were measured during recovery. Bacteria presumptively identified as *Shewanella putrefaciens* and *Pseudomonas putida* were more sensitive to heat than other bacteria, suggesting that most heat treatments of fish may reduce the number of these bacteria. After dissolved oxygen was used up during recovery in nutrient broth, Eh declined from positive to negative values typical of facultative bacteria showing that, after initial aerobic growth, the bacteria changed their metabolism to grow anaerobically. When dissolved oxygen reached zero, about 120 h after inoculation, the numbers of all bacteria were about $10^7 mL^{-1}$, and corresponds to a minimum in the pH values, except for the most heat sensitive isolate. Differential plating showed increased sensitivity to NaCl induced by heat treatments in some isolates (Vaz-Pires *et al*., 1996).

2.2 STAPHYLOCOCCI

Staphylococci, particularly *S.epidermidis,* are members of the normal flora of the human skin and respiratory and gastrointestinal tracts. Nasal carriage of *S.aureus* occurs *in 40- 50* %of human's staphylococci are also found regularly on clothing, bed linens, and other fomites in human environments. The staphylococci are Gram positive spherical cells, usually arranged in grape –like, in irregular clusters. Single cocci, pair's tetrads, and chains are also seen in liquid cultures. Staphylococci are non motile and do not form spores (Geo,Janetand Stephen. 2001).

Staphylococci grow readily on most bacteriologic media under aerobic or microaerophilic condition. They grow most rapidly at 37°C but form pigment best at room temperature. Colonies on solid media are round, smooth, raised, and glistening. *S.aureus* usually forms gray to deep golden yellow colonies. *S. epidermidis* colonies usually are gray to white on primary isolation. No pigment is produced anaerobically or in broth media.

The staphylococci produce catalase which differentiates them from streptococci. Staphylococci slowly ferment many carbohydrates, producing lactic acid but not gas.

The staphylococci are resistant to drying, heat (they withstand 50°C for 30 minutes), and sodium chloride but are readily inhibited by certain chemicals (3% hexachlorophene).

Staphylococci are variably sensitive to many anti microbial drugs. Resistance falls into several classes:

1. ß-lactamase production is common.

2. Resistance to nafcillinis independent of ß-lactamase production.

3. *S.aureus* strains of intermediate susceptibility to vancomycin have been isolated.

4. Plasmids can also carry genes for resistance to tetracycline, erythromycin. All but very few strains of staphylococci have remained susceptible to vancomycin.

5. Tolerance that are inhibited by drug s but not killed by it.

2.2.1 PATHOGENESIS

The pathogenic capacity of a given strain of *S.aureus is* the combined effect of extracellular factors and toxins together with the

invasive properties of the strain. At one end of the disease spectrum is staphylococcal food poisoning, attributable solely to the ingestion of preformed enterotoxin. The potential contribution of the various extracellular substances in pathogenesis is evident from the nature of their individual actions pathogenic invasive *S.aureus* produces coagulase and tends to produce a yellow pigment and to be hemolytic. Non pathogenic, noninvasive staphylococci such as *S.epidermidis* are coagulase- negative and tend to be nonhemolytic.

*Staphlyococcus saprophytic*us is typically nonpigmented, novobiocin- resistant, and non hemolytic, it causes urinary tract infections in young women.

2.2.2 PATHOLOGY

The prototype of a staphylococcal lesion is the furuncle or other localized abscess. Group of *S.aureus* established in a hair follicle lead to tissue necrosis. Coagulate is produced and coagulates fibrin around the lesion and within the lymphatic's, resulting in formation of a wall that limits the process and is reinforced by the accumulation of inflammatory cells and later, fibrous tissue within the center of the lesion, liquefaction of the necrotic tissue occurs and the abscess "points" in the direction of least resistance. Drainage of the liquid center necrotic tissue is followed by slow filling of the cavity with granulation tissue and eventual healing.

Staphylococci also cause disease through the elaboration of toxins, without apparent invasive infection. Bulbous exfoliations, the scalded skin syndrome, is caused by the production of exfoliative toxin. Toxic shock syndrome is associated with toxic shock syndrome toxin-1 (TSST-1).

2.2.3 DIAGNOSTIC LABORATORY

Specimens: surface swab, pus, blood, tracheal aspirate, or spinal fluid for culture, specimens planted on blood agar plates give rise to typical colonies in 18 hours at 37˚C, but hemolysis and pigment production may not occur until several days later and are optimal at room temperature.

S.aureus but not other staphylococci ferment mannitol. And production of catalase and coagulase test must be done on Mac Conkey agar. Smaller colonies are produced after overnight incubation at 35-37˚C. Most strain is non lactose fermenter.

2.2.4 TREATMENT AND PREVENTION

Serious *S.aureus* infections require aggressive treatment, including incision and drainage of localized lesions, as well as systemic antibiotics. There is no effective vaccine against *S.aureus*. Infection control procedures, such as barrier precautions and disinfection of hands and fomites, are important in the control of nosocomial *S.aureus* epidemics (Richard,Harvey,Pamela,Champe and Bruce. 2007).

2.3 Mannheimia

Pasteurella species are primarily animal pathogens, but can produce a range of human diseases. Pasteurellae are non motile Gram-negative coccobacilli with bipolar appearance on stained smears they are aerobes or facultative anaerobes that grow at 37˚C, they are oxidase positive, catalase positive. *Pasteurella haemolytica* occurs in the upper respiratory tract of cattle, sheep, swine and horses. It is a prominent cause of epizootic pneumonia in cattle and sheep and fowl cholera in chickens

(Carter, William and Rikihisa .1986). Human infection appears to be rare. *P.pneumotropica* is a normal inhabitant of the respiratory tract and gut of mice.

2.3.1 LABORATORY DIAGNOSES

Direct examination is of limited value. Media containing serum or blood are required for good growth.Colonies is round, grayish, and usually smaller than *P.multocida* (Carter *et al*., 1986).

2.3.2 EPIDEMIOLOGY

The majority of pasteurella infections in humans are soft tissue infections that follow an animal bite or cat scratch. Smaller fraction of human pasteurella infections occur following a non –bite animal exposure. The source of pasteurella in the latter infections is suspected to be nasopharyngeal colonization of the patient. Laboratory identification by culturing the organisms on blood agar show small, translucent none hemolytic colony.

2.3.3 TREATMENT

Fire soft tissue infections, wounds should be cleansed, irrigated, and dried. Deep- seated infections require surgical drainage and prolonged antibiotic treatment. Penicillin is the drug of choice. Fatal infections are uncommon and usually reflect underlying compromise (Richard *et al*., 2007).

2.4 BACILLUS

The Gram-positive spore-forming bacilli are the bacillus and clostridium species. These bacilli are ubiquitous, and because they form spores they can survive in the environment for many years. Bacillus species are aerobes, Gram- positive rods occurring in chains. Most

members of this genus are saprophytic organisms prevalent in soil, water and air.

The typical cells are arranged in long chains; spore may be central, sub terminal, or terminal, depending on the genus. Colonies of *B.anthracis* are round and have "cut glass" appearance in transmitted light. Hemolysis is uncommon with *B.anthracis* but common with saprophytic bacilli. Gelatin is liquefied, and growth in gelatin stabs resembles an inverted fir tree. The saprophytic bacilli utilize simple sources of nitrogen and carbon of energy and growth. The spores are resistant to environmental changes withstand dry heat and certain chemical disinfectants for moderate periods *B.cereus* cause food poisoning has two distinct forms the emetic type, associated with fried rice, and diarrheal type associated with meat dishes and sauces. *B.cereus* is a soil organism that commonly contaminates rice. And it produces toxin during log-phase growth or during sporulation. A concentration of 10^5 *bacteria* or more per gram of food is considered diagnostic. Also it causes eye infections severe keratitis, and systemic infections (Geo *et al*., 2001).

2.4.1 PATHOLOGY

In susceptible animals, the organisms proliferate at the site of entry. The capsules remain intact, and the organisms are surrounded by a large amount of proteinac-

eous fluid containing few leukocytes from which they rapidly disseminate and reach the blood- stream. In resistant animals, the organisms proliferate for a few hours, by which time there is massive

accumulation of leukocytes. The capsules gradually disintegrate and disappear. The organisms remain localized.

2.4.3 DIAGNOSTIC LABORATORY TESTS

Specimens to be examined are fluid or pus from local lesion, blood, and sputum. Make smear and show chains of large Gram- positive rods.

2.4.4 EPIDEMIOLOGY, PREVENTION, AND CONTROL

Soil is contaminated with anthrax spores from the carcasses of dead animals. These spores remain viable for decades.

Contact with infected animals or with their hides, hair and bristle is the source of infection in human. Control measures include:

1. Disposal of animal carcasses by burning or by deep burial in lime pits.
2. Decontamination of animal products.
3. Protective clothing and gloves for handling potentially infected material.
4. Active immunization of domestic animals with live attenuated vaccine. (Richard *et al*., 2007).

2.5 ENTEROBACTER

Enterobacter organisms are Gram -negative motile rods they can be found in the intestinal tract of humans and animals, and in soil, sewage, water, and dairy products. Enterobacter species are opportunistic pathogens. They are associated with urinary tract infections, wound infection. On blood agar they produce large colonise that may resemble those produced by *klebsiellae* but not so mucoid and non motile (Geo *et al.,* 2001).

2.6 AEROMONAS

Aeromonodas are motile and their colony morphology is similar to that of enteric Gram-negative rods. They produce large zones of heamolysis on blood agar. *Aeromonas species* are distinguished from the enteric Gram-negative rods by finding appositive oxidase reaction in growth obtained from blood agar plate. *Aeromonas species* are differentiated from *vibrios* by showing resistance to compound O/ 129 and lack of growth on media containing 6% Nacl. Typically, Aeromonads produce hemolysins. Some strains produce an enterotoxin. Cytotoxins and the ability to invade cells in tissue culture have been noted.however; none of these characteristics have been clearly shown to be associated with diarrheal disease in humans (Geo *et al*., 2001).

2.7 PROTEUS

Proteus species are relatively common causes of uncomplicated as well as nosocomial urinary tract infections. Other extra intestinal infections, such as wound infections, pneumonias, and septicemias, are associated with compromised patients. *Proteus* organisms produce urease, which catalyzes the hydrolysis of urea to ammonia. The resulting alkaline environment promotes the precipitation of struvite stones containing insoluble phosphates of magnesium and phosphate. (Richard *et al*., 2007).

2.8 ENTEROCOCCI

Enterococci contain a C-carbohydrate that reacts with group D anti sera. Therefore, in the past, they were considered group D streptococci. *Enterococci* can be α - , ß- , or non hemolytic. As a rule, *enterococci* are

not very virulent, but they have become prominent as cause of nosocomial infections as result of their multiple antibiotic resistance.

2.8.1 EPIDEMIOLOGY

Enterococci are part of the normal fecal flora. However, they can also colonize oral mucous membranes and skin, especially in hospital settings. These organisms are highly resistant to environmental and chemical agents, and can persist on fomites. Enterococci seldom cause disease in normal, healthy individuals. However, under conditions in which host resistance is lowered or the integrity of the gastrointestinal or genitourinary tract has been disrupted, enterococci can spread to normally sterile sites, causing urinary tract infections, bacteriamia-sepsis, sub acute bacterial endocarditic.

2.8.2 LABORATORY IDENTIFICATION

Enterococci are distinguished from the non- group D streptococci by their ability to survive in the presence of bile, and to hydrolyze the polysaccharide esculin. Unlike nonenterococcal group streptococci, enterococci grow in 6.5 percent NaCl, and yield a positive pyrazin amidase (PYR) test. *E. faecalis* can be distinguished from *E.faecium* by their fermentation patterns, which are commonly evaluated in clinical laboratories.

2.8.3 TREATMENT

Enterococci are naturally resistant to ß-lactam antibiotics and amino glycosides, but are sensitive to the synergistic action of a combination of these classes. Isolates frequently have natural or acquired resistances to many other antibiotic classes, including glycopeptides, such as

vancomycin. However, some enterococcal strains are resistant to all commercially available antibiotics.

2.8.4 PREVENTION

The rise of nosocomial infections by multiple drugs- resistant enterococci is largely the result of selection due to high antibiotic usage in hospitals. Judicious use of antibiotics is an important factor in controlling the emergence of these infections (Richard *et al*., 2007).

2.9 VIBRIOS

The vibrios are found in marine and surface water. *Vibrio cholerae* produces an enterotoxin that causes cholera a profuse watery diarrhea that can rapidly lead to dehydration and death. Vibrios are among the most common bacteria in surface water worldwide. They are curved aerobic rods and are actively motile possessing a polar flagellum. On prolonged cultivation vibrios may become straight rods that resemble the Gram-negative enteric bacteria. It produce convex, smooth, round colonies that are opaque and granular in transmitted by light grow well at 37 ˚C on many kinds of media including defined media containing mineral salts and asparagines as source of carbon and nitrogen . *V.cholera* grows well on thiosulfate citrate bile sucrose agar (TCBS) and produce yellow colony. Vibrio are oxidase positive and regularly ferments sucrose and mannose but not arabinose. Vibrio species are susceptible to the compound O/129 (2, 4-diamino-6,7-diisopropylpteri-

dine phosphate) which differentiates them from aeromonas species which are resistant to O/129 most vibrio species are halo tolerant and NaCl stimulates their growth. Another difference between aeromonas and

vibrio is that vibrio grow on media containing 6% Nacl whereas Aermonas does not.

2.9.1 Vibrio parahaemolyticus

Is halophilic also it causes acute gastroenteritis following ingestion of contamin-

ated seafood such as raw fish or shellfish. After incubation period of 12-24 hours, nausea and vomiting, abdominal cramps, fever watery diarrhea occurs. faecal leukocytes are observed. No enterotoxin has yet been isolated from organisms. *Vibrio parahaemolyticus* does not grow well on some differential media used to grow salmonella and shigella, but it does grow well on blood agar and characterized by requirement for higher than usual concentration of NaCl and their ability to grow in ten percent NaCl they are common in coastal sea waters.

2.9.2 Vibrio metschnikovi

Resembles *V.cholerae,* has been recovered on several occasions from chickens with enteritis in Europe. The organism is aerobic and grows readily at room temperature on unenriched media or blood agar. The colonies are small, round, yellowish and glistening. Stained smears reveal small, Gram-negative curved rods not unlike other vibrio. It is identified on the basis of growth and biochemical characteristics (Carter *et al.,* 1986).

2.9.3 EPIDEMIOLOGY

Vibrio cholera is transmitted by contaminated water and food there are no known animal reservoirs or animal or arthropod vectors there are two biotypes of the species *V.cholerae* classic and Eltor, The Eltor strain is distinguished by the production of haemolysin and ability to survive in

water for longer periods. Out breaks of both strains have been associated with raw or under cooked sea food harvested from contaminated waters.

2.9.4 PATHOGENESIS AND PATHOLOGY

Under natural conditions, *V.cholera* is pathogenic only for humans, person with normal gastric acidity may have to ingest as many as 10 or more. *V.cholerae* to become infected when the vehicle is water when the vehicle is food, as few as 10^2 -10^4 is necessary because of the buffering capacity of food.

Cholera is not an invasive infection. The organisms do not reach the blood stream but remain within the intestinal tract. *V.cholerae* attach to microvilli of the brush border of epithelial cell there they multiply and liberate cholera toxin and perhaps mucinasas and endotoxin.

2.9.5 DIAGNOSTIC LABORATORY TESTS

Specimen for culture mucus flecks from stools Growth is rapid in peptone agar, on blood agar with pH near 9.0 or on TCBS agar also on Mac Conkey agar and typical colonies are picked in 18 hours.

2.9.6 TREATMENT AND PREVENTION

The most important part of therapy consists of water and electrolyte replacement to correct the severe dehydration and salt depletion and any antimicrobial agents are effective against *V. cholerae.* Oral tetracycline tends to reduce stool output in cholera and shorten the period of excretion of vibrios (Richard *et al*., 2007).

2.10 CITROBACTER

The influence of the growth medium and the growth temperature on the heat resistance of *Citrobacter freundii* has been established.

Logarithmic growth phase cells grown on rich media have a higher heat resistance than cells of the same phase grown on minimal media. This finding was independent of type of carbon source in the growth medium, but the kind of carbon source has a definite influence on the heat resistance. Logarithmic phase cells grown at 37°C are much more heat stable than cells grown at 20 or 41°C. Stationary growth phase cells are much more heat resistant than logarithmic phase cells, whereas Mg^{2+}-or glucose-starved cells are even slightly more heat stable than stationary phase cells (Verrips,Kawast and Devries. 1980).

2.11 KURTHIA

Gram-positive rods in chains, motile but non motile variants occur, no acid –fast, strictly aerobic. Acid not produced from sugars in peptone media, acetoin not produced, nitrate not reduced. It is not usually regarded as a pathogen, but we include it as strains have been isolated from meat and dairy products and occasionally from clinical material (Keddie, 1981).

Surface colonies are rhizoid but unlikely to be confused with *Bacillus* species: they are not haemolytic. Two species, *K .zopfii* and *K.gibsonii* are recognized though other psychrophilic strains occur with many kurthia – like characters (Shaw and Keddie, 1983, Keddie and Shaw, 1986). Growth occurs in the range -5 ˚to 35˚C for *K.zopfii* and -5˚ to 45˚C or more for *K.gibsonii.* They give negative reactions in most of the usual biochemical tests. The bird's feather growth on nutrient gelatin slopes is highly distinctive for both species (Barrow and Feltham, 1993).

2.12 *Stomatococcus mucilaginosus*

An oral commensal organism in man may be associated with occasional opportunistic infections. It can be recognized by the sticky adherent nature of colonies on solid media and by positive aesculin reaction (Barrow and Feltham, 1993).

2.13 MICROCOCCUS

Micrococcus is a genus of bacteria in the Micrococcaceae family. *Micrococcus* occurs in a wide range of environments, including water, dust and soil. Micrococci have Gram-positive spherical cells ranging from about 0.5 to 3 micrometers in diameter and are typically appear in tetrads. *Micrococcus* has a substantial cell wall, which may comprise as much as 50% of the cell mass. The genome of *Micrococcus* is rich in guanine and cytosine (GC), typically exhibiting 65 to 75% GC-content. Micrococci often carry plasmids (ranging from 1 to 100MDa in size) that provide the organism with useful traits (Doddaman and Ninnekar. 2001).

2.13.1 ENVIRONMENTAL

Micrococci have been isolated from human skin, animal and dairy products, and beer. They are found in many other places in the environment, including water, dust and soil. *M. luteus* on human skin transforms compounds in sweat into compounds with an unpleasant odor. Micrococci can grow well in environments with little water or high salt concentrations. Most are mesophiles , some like *Micrococcus antarcticus* (found in Antarctica) are psychrophiles. Though not a spore former, *Micrococcus* cells can survive for an extended period of time. Unprotected cultures of soil micrococci have been revived after storage in a refrigerator for 10 years. Demonstrate that *Micrococcus luteus* has

survived for at least 34,000 to 170,000 years on the basis of 16S r RNA analysis, and possibly much longer (Greenblat, Baum, Klein, Nachshon, Koltunov and Cano. 2004).

2.13.2 PATHOGENESIS

Micrococcus is generally thought to be a saprotrophic or commensal organism, though it can be an opportunistic pathogen, particularly in hosts with compromised immune systems, such as HIV patients (Smith, Neafie,Yeager andSkelton. 1999). It can be difficult to identify *Micrococcus* as the cause of an infection, since the organism is a normally present in skin micro flora, and the genus is seldom linked to disease. In rare cases, death of immunocompromised patients has occurred from pulmonary infections caused by *Micrococcus*. Micrococci may be involved in other infections, including recurrent bacteremia, septic shock, septic arthritis, endocarditis, meningitis, and cavitating pneumonia (immunosuppressed patients).

*2.*14 *Escherichia coli*

E. coli is Gram-negative, facultative anaerobic and non-sporulating. The cells are about 2 micrometres (μm) long and 0.5 μm in diameter, with a cell volume of 0.6 - 0.7 μm (Kubitschek, 1990). It can live on a wide variety of substrates. *E. coli* uses mixed-acid fermentation in anaerobic conditions, producing lactate, succinate, ethanol, acetate and carbon dioxide. Since many pathways in mixed-acid fermentation produce hydrogen gas, these pathways require the levels of hydrogen to be low, as is the case when *E.coli* lives together with hydrogen-consuming organisms such as methanogens or sulfate-reducing bacteria (Madigan and Martinko, 2006).

Optimal growth of *E. coli* occurs at 37°C, but some laboratory strains can multiply at temperatures of up to 49°C (Fotadar,Zaveloff andTerracio. 2005). Growth can be driven by aerobic or anaerobic respiration, using a large variety of redox pairs, including the oxidation of pyruvic acid, formic acid, hydrogen and amino acids, and the reduction of substrates such as oxygen, nitrate, dimethyl sulfoxide and rimethylamine N-oxide (Ingledew and Poole,1984). Strains that possess flagella can swim and are motile, but other strains lack flagellum. The flagella of *E. coli* have a peritrichous arrangement (Darnton, Turner, Rojevsky and Berg . 2007).

Escherichia coli and related bacteria possess the ability to transfer DNA via bacterial conjugation, transduction or transformation, which allows genetic material to spread horizontally through an existing population. This process led to the spread of the gene encoding shiga toxin from *Shigella* to *E.coli* O157:H7, carried by a bacteriophage.

2.14.1 GASTROINTESTINAL INFECTION

Low-temperature electron micrograph of a cluster of *E. coli* bacteria, magnified 10,000 times. Each individual bacterium is oblong shaped.

Certain strains of *E. coli*, such as O157:H7, O121 and O104:H21, produce toxins. Food poisoning caused by *E. coli* is usually associated with eating unwashed vegetables and meat contaminated post-slaughter. O157:H7 is further notorious for causing serious and even life-threatening complications like hemolytic-uremic syndrome (HUS). This particular strain is linked to the 2006 United States *E. coli* outbreak of fresh spinach. Severity of the illness varies considerably, it can be fatal, particularly to young children, the elderly or the immunocompromised,

but is more often mild. *E. coli* can harbor both heat-stable and heat-labile enterotoxins. The latter, termed LT, contains one 'A' subunit and five 'B' subunits arranged into one holotoxin, and is highly similar in structure and function to Cholera toxins. The B subunits assist in adherence and entry of the toxin into host intestinal cells, while the A subunit is cleaved and prevents cells from absorbing water, causing diarrhea. LT is secreted by the Type 2 secretion pathway.

If *E. coli* bacteria escape the intestinal tract through a perforation and enter the abdomen, they usually cause peritonitis that can be fatal without prompt treatment. However, *E. coli* are extremely sensitive to such antibiotics as streptomycin or gentamicin. This could change since, as noted below, *E. coli* quickly acquires drug resistance. Recent research suggests that treatment with antibiotics does not improve the outcome of the disease, and may in fact significantly increase the chance of developing haemolytic uraemic syndrome (Wong,Jelacic andHabeeb. 1930).

Intestinal mucosa-associated *E. coli* are observed in increased numbers in the inflammatory bowel diseases, Crohn's disease and ulcerative colitis (Roihion and Darfeuille ., 2007). Invasive strains of *E. coli* exist in high numbers in the inflamed tissue, and the number of bacteria in the inflamed regions correlates to the severity of the bowel inflammation (Baumgart,Dogan and Rishniw. 2007).

2.14.2 VIRULENCE PROPERTIES

Enteric *E. coli* (EC) are classified on the basis of serological characteristics and virulence properties (Todar, 2007). Virotypes include:

- **Enterotoxigenic *E. coli*** (ETEC): causative agent of diarrhea (without fever) in humans, pigs, sheep, goats, cattle, dogs, and horses. ETEC uses fimbrial adhesions (projections from the bacterial cell surface) to bind enterocyte cells in the small intestine. ETEC can produce two proteinaceous enterotoxins: the larger of the two proteins, LT enterotoxin, is similar to cholera toxin in structure and function, while the smaller protein, ST enterotoxin causes cGMP accumulation in the target cells and a subsequent secretion of fluid and electrolytes into the intestinal lumen. ETEC strains are non-invasive, and they do not leave the intestinal lumen. ETEC is the leading bacterial cause of diarrhea in children in the developing world, as well as the most common cause of traveler's diarrhea. Each year, ETEC causes more than 200 million cases of diarrhea and 380,000 deaths, mostly in children in developing countries.

- **Enteropathogenic *E. coli*** (EPEC): causative agent of diarrhea in humans, rabbits, dogs, cats and horses. Like ETEC, EPEC also causes diarrhea, but the molecular mechanisms of colonization and etiology are different. EPEC lack fimbriae, ST and LT toxins, but they utilize an adhesin known as intimin to bind host intestinal cells. This virotype has an array of virulence factors that are similar to those found in *Shigella*, and may possess a shiga toxin. Adherence to the intestinal mucosa causes a rearrangement of actin in the host cell, causing significant deformation. EPEC cells are moderately-invasive (i.e. they enter host cells) and elicit an inflammatory response. Changes in intestinal cell ultra structure

due to "attachment and effacement" is likely the prime cause of diarrhea in those afflicted with EPEC.

- **Enteroinvasive *E. coli*** (EIEC): found only in humans. EIEC infection causes a syndrome that is identical to Shigellosis, with profuse diarrhea and high fever. EIEC are highly invasive, and they utilize adhesin proteins to bind to and enter intestinal cells. They produce no toxins, but severely damage the intestinal wall through mechanical cell destruction.

- **Enterohemorrhagic *E. coli*** (EHEC): found in humans, cattle, and goats. The sole member of this virotype is strain O157:H7, which causes bloody diarrhea and no fever. EHEC can cause hemolytic-uremic syndrome and sudden kidney failure. It uses bacterial fimbriae for attachment, is moderately-invasive and possesses a phage-encoded Shiga toxin that can elicit an intense inflammatory response.

- **Enteroaggregative *E. coli*** (EAggEC): found only in humans. So named because they have fimbriae which aggregate tissue culture cells, EAggEC bind to the intestinal mucosa to cause watery diarrhea without fever. EAggEC are non-invasive. They produce a hemolysin and an ST enterotoxin similar to that of ETEC.

2.14.3 EPIDEMIOLOGY OF GASTROINTESTINAL INFECTION

Transmission of pathogenic *E. coli* often occurs via fecal-oral transmission (Gehlbach, MacCormack, and DrakeandThompson.1973). Common routes of transmission include: unhygienic food preparation,

farm contamination due to manure fertilization (Sabin, 2006). Irrigation of crops with contaminated grey-water or raw sewage (Heaton and Jones, 2007). Feral pigs on cropland, (Thomas, 2007). Or direct consumption of sewage-contaminated water (Chalmers,Aird and Bolton. 2000). Dairy and beef cattle are primary reservoirs of *E. coli* O157:H7 (Bach,McAllister,Veira,Gannon and Holley.2002). And they can carry it asymptomatically and shed it in their feces. Food products associated with *E. coli* outbreaks include raw ground beef, raw seed sprouts or spinach (Sabin, 2006). Raw milk, unpasteurized juice, and foods contaminated by infected food workers via fecal-oral route.

According to the U.S. Food and Drug Administration, the fecal-oral cycle of transmission can be disrupted by cooking food properly, preventing cross-contamination, instituting barriers such as gloves for food workers, instituting health care policies so food industry employees seek treatment when they are ill, pasteurization of juice or dairy products and proper hand washing requirements.

Shiga toxin-producing *E. coli* (STEC) specifically serotype O157:H7, have also been transmitted by flies (Szalanski,Owens,McKayand Steelman. 2004). As well as direct contact with farm animals (Rahn *et al.*, 1998 and Trevena *et al*., 1999). Petting zooanimals, (Heuvelink *et al*., 2002) and airborne particles found in animal-rearing environments (Varma *et al*., 2003).

2.14.4 LABORATORY DIAGNOSIS

In stool samples microscopy will show Gram-negative rods, with no particular cell arrangement. Then, either MacConkey agar or EMB agar (or both) are inoculated with the stool. On MacConkey agar, deep red

colonies are produced as the organism is lactose positive, and fermentation of this sugar will cause the medium's pH to drop, leading to darkening of the medium. Growth on Levine EMB agar produces black colonies with greenish-black metallic sheen. This is diagnostic of *E. coli*. The organism is also lysine positive, and grows on TSI slant with a (A/A/g+/H_2S-) profile. Also, IMViC is positive for *E. coli*, as its indol positive, and methyl red positive, but VP negative and citrate negative. Tests for toxin production can use mammalian cells in tissue culture, which are rapidly killed by shiga toxin. Although sensitive and very specific, this method is slow and expensive (Paton and Paton. 1998).

Typically diagnosis has been done by culturing on sorbitol-MacConkey medium and then using typing antiserum. However, current latex assays and some typing antiserum have shown cross reactions with non-*E.coli* O157 colonies. Furthermore, not all *E.coli* O157 strains associated with HUS are non sorbitol fermentors.

The Council of State and Territorial Epidemiologists recommend that clinical laboratories screen at least all bloody stools for this pathogen. The American Gastroenterological Association Foundation (AGAF) recommended in July 1994 that all stool specimens should be routinely tested for *E.coli* O157:H7. It is recommended that the clinician check with their state health department or the Centers for Disease Control and Prevention to determine which specimens should be tested and whether the results are reportable.

Other methods for detecting *E. coli* O157 in stool include ELISA tests, colony immunoblots, direct immunofluorescence microscopy of filters, as well as immunocapture techniques using magnetic beads (De

Boer and Heuvelink, 2000). These assays are designed as screening tool to allow rapid testing for the presence of *E. coli* O157 without prior culturing of the stool specimen.

CHAPTER THREE
MATERIALS AND METHODS

3.1COLLECTION OF SAMPLES

Mutton and beef were collected from butcher's shops and camel meats from slaughter house in Khartoum state. The following sites were chosen for sampling: neck, top site, Loin, sirloin, thin flank and shank.

Meat samples were collected using sterile scissors and put in sterile Petri dishes and transported immediately to the laboratory. Cultures were carried out within less than 5 hours. Samples were cultured on blood agar and incubated over night at 37˚C. Gram stain was performed according to Barrow and Feltham (2003), primary tests; secondary tests were carried out to identify the bacteria isolated.

3.2CULTURAL MEDIA

3.2.1 NUTRIENT AGAR

A rehydrated form of medium was prepared. 28 grams of powder were dissolved in 1000 milliliter of distilled water into flask then boiled in water bath and sterilized by autoclaving at 121°C under pressure 15 pounds per square inch for 15minutes after that the medium was distributed into sterile Petri dishes.

3.2.2 BLOOD AGAR

Nutrient agar (Oxoid) was rehydrated. Twenty eight grams of powder were added to1000 milliliter of distilled water and boiled to dissolve. The pH 7.2. Then autoclaved at121°C under 15pounds per square inch for 15 minutes.

To prepare blood agar plates the citrated sheep blood was added aseptically to the nutrient agar as base media after it was cooled and mixed gently and poured onto sterile Petri dishes.

3.2.3 AESCULIN AGAR

One gram of aesculin, 0.5 gram ferric citrate and 20 grams of agar were dissolved in 100 milliliter peptone water and sterilized at 115°C for ten minutes under pressure of ten pounds per square inch. The melted medium was poured aseptically into sterile Mc Cartney bottles and allowed to set in the slope position.

3.2.4 SIMMON'S CITRATE AGAR

Twenty grams of dehydrated medium were dissolved by boiling in1000 milliliter of distilled water and sterilized by autoclaving at 121°C for15 minutes. Sterilization was done under pressure of 15 pounds per square inch. The sterilized medium was poured aseptically into sterile McCartney bottles and allowed to set in the slope position.

3.2.5 UREA MEDIUM OR CHRISTENSEN'S UREA MEDIUM

This medium was obtained from Oxoid and prepared according to the instructions. Two point four grams of urea agar base were dissolved in 95 milliliter of distilled water by heat. And pH was checked (7.4) then sterilized at 115°C for 20minutes. The medium was cooled to50°C then five milliliter of sterile 40% urea solution was added aseptically and mixed well and distributed into sterile McCartney's bottles and allowed to set in the slope position.

3.2.6SEMI SOLID MEDIA

3.2.6.1 MOTILITY MEDIUM

Contains 0.2 percent New Zealand agar,was dissolved in nutrient broth and distributed in sterile test tubes containing Graigies tubes, then the medium was autoclaved at 121°C for 15 minutes.

3.2.6.2 HUGH AND LEIFSON'S (O-F) MEDIUM

The ingredients of medium: Five grams of sodium chloride (NaCl),0.3 grams of dipotassium hydrogen phosphate (K2HPO4), two grams of peptone, and three grams agar. These solid ingredients were dissolved in 1000 milliliters distilled water. The pH was adjusted to 7.1 and 15 milliliter of two percent aqueous solution bromothymol blue was added to the medium.

Sterile solution of glucose was added to the medium to give a final concentration of one per cent. The medium was mixed and distributed in test tubes pluged with cotton and sterilized by autoclaving.

3.2.8 SUGAR MEDIA

Nine hundred milliliters of the base medium (Smith Holdeman**'**s medium) with pH 7.1,10 milliliter Andrade's indicators and 10 grams sugar were added. The medium was poured into test tubes containing Durham's tubes. The mixture was sterilized by autoclaving at 115˚ C for 15minutes.

3.2.9 METHYL RED AND VOGES-PROSKAUER MEDIUM (MR AND V-P MEDIUM)

Five grams of peptone and five grams of potassium hydrogen phosphate were dissolved in1000 milliliter distilled water, then five

grams of glucose were added to the medium and the mixture was distributed into tubes and sterilized at 115˚ C for15minutes.

3.2.10 NUTRIENT BROTH

Thirteen grams of dehydrated nutrient broth were added to1000 milliliter of distilled water. The medium was thoroughly mixed, and sterilized at 121˚ C for 20 minutes.

3.2.11 ARGININE BROTH

The medium was prepared according to the formula given by Barrow and Feltham(1993) . It contains five grams peptone, five grams yeast extract, two grams dipotassium hydrogen phosphate (K_2HPO_4), 0.5 grams glucose and three grams arginine monohydrochloride which were dissolved in 1000 milliliter distilled water by boiling. The medium was adjusted to pH 7.0 and sterilized at 115◦C and pressure of ten pounds per square inch for 20 minutes.

3.2.12 NITRATE BROTH

One gram of potassium nitrate (KNO_3) was dissolved in 1000 milliliter nutrient broth. The medium was distributed into test tubes and sterilized at 121◦Cfor 15 minutes.

3.2.13 PEPTONE WATER

Five grams of peptone and five grams of sodium chloride were dissolved in 1000millilitree of distilled water by boiling. The pH was adjusted to 7.2- 7.4 and then autoclaved at 121◦C for 20 minutes

3.3 REAGENTS

Reagents were prepared according to Barrow and Feltham (2003).

3.3.1NESSLER'S REAGENT

Five grams of potassium iodide were dissolved in five milliliter freshly distilled water. Cold saturated mercuric chloride solution was added to the solution until slight precipitate remained permanently after thoroughly shaking. Forty milliliter 9-N sodium hydroxide was added. The mixed solution was diluted into 100 milliliter distilled water and allowed to stand for 24 hours before use.

3.3.2 OXIDASE TEST REAGENT

One per cent tetra methyl – p-phenylenediamine aqueous solution was added to one per cent ascorbic acid. Filter paper of 5 × 50 millimeter size were impregnated in the above reagent and dried at 50˚C.

3.3.3 NITRATE REAGENTS

Reagents prepared in the laboratory. Nitrate reagents (solution A and solution B) were used. Ingredientsof solution A:0.8 % sulphanilic acid was dissolved by gentle heating in 5N- acetic acid. Ingredient of solution B: 0.6 % dimethyl –α naphthylamine in 5 N–acetic acid using heat.

3.3.4 HYDROGEN PEROXIDE

Hydrogen peroxide 3% solution was diluted to prepare solution for catalase test.

3.3.5 METHYL RED

Readily prepared in the lab, 5%aqueous solution for use in MR test.

3.3.6 KOVAC'S REAGENT

The reagent was prepared as described by Barrow and Feltham(2003). Five grams of p- dimethylaminobenzaldehyde were dissolved in 75 milliliter of amyl alcohol by warming in a water bath.

Twenty –five milliliter of concentrated hydrochloric acid was added to the mixture after it was cooled.

3.4 METHODS OF STERILIZATION

3.4.1DRY HEAT

Hot air oven: was used for sterilization of glass ware. The temperature and time of exposure were 160°C for 60 minutes.

Red heat: it was used for sterilization of the wire loops, straight wires, it was done by holding the object as near vertical as possible until became red-hot.

Flaming: this method was used for the cotton plugged tubes openings with cotton plugs, and glass slides. It was done by exposing the object to the flame for a few second.

Moist heat: autoclaving: this device was used for sterilization of cultural media, the temperature of autoclaving was 115-121 °C, the holding time was 15 to 20 minutes and the pressure was 10 to 15 pounds per square inch gauge pressure.

3.5 METHODS FOR BACTERIAL ISOLATION AND IDENTIFICATION

After over night incubation period was finished, Gram stain was applied out, thin smears of each isolate were fixed by heat and then flooded with crystal violet for one minute, washing off with water, flooded again with lugol's iodine for one minute. The slide was then rinsed with water, decolorized with acetone that was washed, immediately. Then flooded with dilute carbol fuchsin for 30 seconds and washed finally the stained film was blotted dry and examined under

immersion oil lens (100×). Pure cultures were obtained by several times subculture of the primary growth.

3.5.1 TESTING FOR MOTILITY OF ISOLATED BACTERIA

The motility of the isolates was tested using the Graigie's technique. Small inoculums of the culture was introduced inside Graigie's tube in semisolid agar media placed into test tube. The test tubes were incubated at 37°C for 24- 48hours. The tubes were examined for migration of the bacteria outside the Graigie's tube.

3.5.2 CATALASE TEST

According to Barrows and Feltham (1993) a drop of hydrogen peroxide (H_2O_2) was placed on a clean slide. Using a sterile wood stick, a small part of an isolated colony was taken and emulsified in the hydrogen peroxide drop, the production of gas bubbles was considered positive reaction.

3.5.3 OXIDASE TEST

The colony to be tested was picked up by glass rod and smeared or spreaded on oxidase paper. Positive reaction was indicated by change in color to the violet.

3.5.4 THE OXIDATION FERMENTATION (OF) TEST

Two test tubes containing (OF) medium were inoculated. One of the two tubes was topped with a layer of liquid paraffin oil layer. The tubes were incubated at 37°C for up to 15 days. And they were examined for change in color. The yellow colour indicates acid production. If the change in the open tube, the reaction oxidative. If the yellow color was in both tubes it was fermentative reaction.

3.5.5 SUGAR REACTIONS

The inoculated tubes containing liquid sugar medium were incubated at 37°C for 24 hours and examined for production of acid or gas. The production of acid was indicated by change in Andrade's indicator to pink rosette color. The presence of empty space in the upper part of the Durham's tube indicated the production of gas.

3.5.6 AESCULIN HYDROLYSIS TEST

Aesculin agar bottles were inoculated with the isolates to be tested and incubated at 37 °C for up to seven days. The inoculated bottles were examined daily for blacken of the medium due to hydrolysis of aesculin.

3.5.7 ARGININE HYDROLYSIS

This test was carried out when five milliliter of arginine broth were inoculated with the isolate to be tested. After 24 hours incubation period at 37 °C, 0.25 milliliter of Nissler's reagent was added. Development of brown color indicated arginine hydrolysis.

3.5.8 NITRATE REDUCTION TEST

Nitrate broth was inoculated with the isolate and incubated at 37°C for five days. Then nitrate reagent A was added followed by nitrate reagent B. Reduction of the nitrate was indicated by the red color development. When there was no change in color a few or a knife point of zinc dust powder was added to the culture. If red color developed the result was negative. If no red color developed it is considered as nitrate positive.

3.5.9 UREASE ACTIVITY TEST

The medium of urea was inoculated with the isolate under test and incubated at 37°C and examined for up to five days for change in color of medium to the pink color.

3.5.10 CITRATE UTILIZATION TEST

The test organism was cultured on surface of Simmon's citrate medium, incubated at 37°C and examined daily up to seven days for change in color to blue which means citrate is utilized.

3.5.11 HYDROGEN SULPHIDE TEST

The isolate to be tested was inoculated in peptone water, then a lead acetate paper was inserted between the tube and cotton and was incubated at 37°C for up to six days. Hydrogen sulphide production was indicated by the blackening of the lead acetate paper.

3.5.12 INDOLE PRODUCTION TEST

Peptone water was inoculated with the organism under test and incubated at 37°C for 48 hours. Then Kovac's reagent was added to the culture, the presence of indole was indicated by development of a red color ring.

3.5.13 METHYL RED (MR) AND VOGUES – PROSKAUR (V-P) TEST

Two tubes of glucose phosphate medium were inoculated with the bacteria under test and incubated for two days at 37°C. A drop of MR reagent was added to culture. Development of red color indicates positive test, the second tube 0.6 milliliter α -naphthol solution and 0.2 milliliter 40% potassium hydroxide aqueous solution were added. The tube was

shaken and sloped , cotton was removed and left for 15 minutes at room temperature development of red pink color .which positive VP.

3.6. THERMAL DEATH OF ISOLATES

Overnight cultures of a particular isolate which was grown in nutrient broth in test tubes and incubated at 37˚C, was immersed into a water bath. Up to the neck of the tube. A drop of culture was removed and grown on blood agar at 37˚Cfor 24 hours temperatures and holding times set shown below. The results were recorded as “growth” or “no growth”.

The following treatments were followed for all isolates:

Temperature ˚C	Holding Time (min)
70 ˚C	30
70˚ C	60
80˚C	15
80˚C	30
90˚C	5
90˚C	10
90˚C	15
100˚C	5
100˚C	10

CHAPTER FOUR
RESULTS

4.1. Isolates

The total number of isolates obtained from the meat samples was 70.

4.2. Identification of Isolates

According to the microscopic, cultural and biochemical activities, these isolates were identified as:*Aeromonas salmonicida,Aeromonas hydrophila, Vibrio metschnikovii, Vibrio cincinnatures, Proteus penneri, Staphylococcus caseolyticus, Micrococcus varians, Kurthia gibsonii, Vibrio para haemolyticus, Enterobacter gergoviae, Bacillus mycodes ,Stomatococcus mucilaginosus, Enterococcus mundtii, Mannheimia haemolytica, Vibrio furnisii, Escherichia coli, Citrobacter freundii.*

Table (1)

Distribution of isolates according to meat type and its site on carcass and source was shown on table (1).

Meat type	Site	Number of sample	Source
Beef	Loin	6	Omdurman
	Sirloin	5	Kh.North
	Neck	4	
Mutton	Neck	3	Omdurman
	Top site	5	Kh.North
	Loin	7	
	Thinflank	7	
	Shank	9	
Camel	Top site	1	Karari slaughter
	Loin	3	
	Thinflank	2	
	sirloin	1	

Mutton was found most contaminant type of meats (7 bacterial species), beef (6bacterial species) and the less contaminant meat type was the camel meat (4bacterial species).

Table (2) Thermal Death of Isolates

All organisms under test were killed by the different temperatures applied except *Bacillus mycoides*, which survived boiling for 10 minutes.

Species	*Aeromonas Salmonicida*.	*Vibrio metschnikovii*	*Vibrio cincinnatures*	*Proteus penneri*	*Staphylococcus caseolyticus*	*Micrococcus varians*	*Kurthia gibsonii*	*Aeromonas hydrophila*
100°c/5 min	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead
90°c/15 min	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead
90°c/10 min	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead
90°c/5 min	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead
80°c/30 min	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead
80°c/15 min	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead
70°c/60 min	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead
°70c/30 min	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead
100°c/10min	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead

Vibrio para haemolyticus	*nterobacter gergoviae*	*Bacillus mycodes*	*Stomatococcus mucilaginosus*	*Enterococcus mundtii*	*Mannheimia haemolytica*	*Vibrio furnisii*	*Escherichia coli*	*Citrobacter freundii*
Dead	Dead	Growth	Dead	Dead	Dead	Dead	Dead	Dead
Dead	Dead	Growth	Dead	Dead	Dead	Dead	Dead	Dead
Dead	Dead	Growth	Dead	Dead	Dead	Dead	Dead	Dead
Dead	Dead	Growth	Dead	Dead	Dead	Dead	Dead	Dead
Dead	Dead	Growth	Dead	Dead	Dead	Dead	Dead	Dead
Dead	Dead	Growth	Dead	Dead	Dead	Dead	Dead	Dead
Dead	Dead	Growth	Dead	Dead	Dead	Dead	Dead	Dead
Dead	Dead	Growth	Dead	Dead	Dead	Dead	Dead	Dead
Dead	Dead	Growth	Dead	Dead	Dead	Dead	Dead	Dead

Table (3) The primary biochemical properties of bacteria isolated from mutton, beef and camel meats.

bacteria isolated	Gram reaction	Motility	Oxidase	Catalase	Glucose	OF
Aeromonas Salmonicida.	**-ve**	-	+	+	+	**F**
Vibrio metschnikovii	**-ve**	-	+	-	+	**F**
Vibrio cincinnatures	**-ve**	+	+	+	+*	**F**
Proteus penneri	**-ve**	+	-	+	+	**F**
Staphylococcus caseolyticus	**-ve**	+	+	+	+	**F**
Micrococcus varians	**-ve**	+	+	+	+*	**F**
Kurthia gibsonii	**-ve**	+	+	+	+	**F**
Aeromonas hydrophila	**-ve**	+	-	+	+	**F**
Vibrio para haemolyticus	**-ve**	-	-	+	+	**F**
Enterobacter gergoviae	**-ve**	+	-	+	+	**F**
Bacillus mycodies	**-ve**	+	-	+	+*	**F**
Stomatococcus mucilaginosus	**+ve**	-	+	+	+	**O**
Enterococcus mundtii	**+ve**	-	-	+	+	**F**
Mannheimia haemolytica	**+ve**	-	+	+	+	**F**
*Vibrio furnisii *	**+ve**	-	-	-	+	**F**
Escherichia coli	**+ve**	+	-	+	-	-
Citrobacter freundii	**+ve**	-	+	+	+	-

* Gas production.

Table (3) Secondary biochemical properties of bacteria species isolated from mutton, beef and camel meat.

No	Man	Ins	Ind	Aes	H_2S	Cit	Ure	Nit	Cell	Ara	Sal	Sor	Vp	Raff	Xyl	Lac	Suc	Fr
1	ND	ND	-	ND	ND	ND	-	+	ND	+	+	-	ND	-	ND	-	-	ND
2	ND	ND	-	+	ND	ND	-	+	ND	+	ND	+	ND	+	-	-	ND	ND
3	ND	ND	-	ND	ND	ND	ND	+	ND	+	-	ND	-	ND	-	ND	+	ND
4	ND	ND	-	ND	ND	ND	ND	-	ND	+	-	ND	-	+	-	ND	+	ND
5	ND	ND	-	+	-	ND	ND	+	ND	ND	+	ND	+	ND	+	ND	+	ND
6	ND	+	+	+	+	ND	ND	+	ND	ND	+	ND	+	ND	ND	ND	+	ND
7	ND	ND	+	ND	ND	ND	+	+	ND	+	-	+	-	ND	-	ND	-	ND
8	ND	-	-	ND	+	-	+	ND	-	-	ND	ND	-	+	ND	-	-	ND
9	+	ND	+	ND	+	-	-	ND	ND	-	ND	+	-	-	+	+	ND	ND
10	ND	-	-	ND	+	+	+	ND	+	ND	+	-	-	ND	+	-	ND	ND
11	-	ND	-	ND	+	+	ND	+	+	ND	ND	ND	+	-	-	+	ND	ND
12	-	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	-	ND	-	+	+	+
13	ND	ND	ND	ND	ND	ND	ND	+	ND	ND	ND	ND	-	ND	ND	ND	+	+
14	ND	ND	ND	ND	ND	ND	ND	+	ND	ND	-	-	-	ND	ND	-	+	+
15	ND	ND	ND	+	ND	ND	ND	ND	ND	+	ND	+	+	ND	ND	+	+	ND
16	ND	ND	-	-	-	-	ND	-	-	ND	ND	ND	ND	ND	ND	-	-	ND
17	ND	-	-	ND	+	+	ND	+	+	ND	+	-	+	ND	ND	-	+	ND

Man = Mannose; Ins = Insitol; Ind = Indole; Aes = Aesculin; H_2S = Hydrogen sulfat; Cit= Citrate; Ure= Urease; Nit = Nitrate; Cell = Cellobiose; Ara=Arabinose; Sal=Salicin; Sor = Sorbitol; VP = Vogues-Proskaur; Raff = Raffinose; Xvl = Xvlose; Lac = lactose; Suc = Sucrose; Fr = Fructose. ND=Not Done

Table (5): The frequency of the isolates according to type of meat and its site.

Bacteria isolated	Meat type	Carcass Site	Frequency
Aeromonas Salmonicida.	Mutton	Shank	2
Vibrio metschnikovii	Mutton	Neck	1
Vibrio cincinnatures	Mutton	Shank	1
Proteus penneri	Mutton	Loin	1
Staphylococcus caseolyticus	Mutton	Neck	1
Micrococcus varians	Mutton	Loin	14
Kurthia gibsonii	Mutton	Loin	13
Aeromonas hydrophila	Beef	Loin	1
Vibrio para haemolyticus	Beef	Sir loin	1
Enterobacter gergoviae	Beef	Topsite	1
Bacillus mycoides	Beef	Neck	9
Stomatococcus mucilaginosus	Beef	Topsite	13
Enterococcus mundtii	Beef	Top site	1
Mannheimia haemolytica	Camel	Topsite	1
*Vibrio furnisii *	Camel	Thin flank	1
Escherichia coli	Camel	Topsite	8
Citrobacter freundii	Camel	Loin	1
Total			70

CHAPTER FIVE
DISCUSSION AND CONCLUSION

Many consumers and cookers are warned against eating uncooked or extremely rare meat, because of associated health risks. Meat is often contaminated with bacteria such as Salmonella and Listeria which can be killed by cooking at a proper temperature. Rare meat may still contain living bacteria which can cause infection resulting in intestinal discomfort and, in rare cases death. If prepared safely, some red meat can be eaten rare, while white meat, including poultry and pork, should never be eaten rare.

Rare meat is meat that has been minimally cooked, leaving the inside still red and tender. For rare meat to be considered safe, it should be heated to at least 140 degrees Fahrenheit (60 Celsius). Meat that is not cooked to this temperature will still contain living bacteria. Meat thermometer must be used to ensure that meat is safe to eat. Consumers should avoid eating raw or extremely rare meat, including steak tartare, because of the risk of infection.

These warnings are substantiated by the present findings presented in this thesis.

Since most bacteria accumulate on the outside of meat, searing the outside of a cut of meat will eliminate most of the risks of eating rare meat. Consumers should be aware that pockets of bacteria may still exist inside rare meat, however, and that for true food safety the meat should

be evenly cooked all the way through. When cooking for pregnant women and people with compromised immune systems, always meat must be cooked thoroughly.

Basic safety procedures should be used when handling any meat, especially rare meat. Meat labels generally indicate what temperature it should be kept at, and meat should be kept under proper refrigeration at all times, or frozen if not being used immediately. Meat should always be prepared separately from other foods. The knives and cutting boards used to prepare meat should be washed and sterilized thoroughly to prevent cross contamination. Leftover cooked meat should be covered and immediately refrigerated or discarded. Since leftovers generally do not keep more than two or three days, they should be eaten quickly. Also, since meat can collect bacteria if reheated, it should be reheated only once.

When meat is exposed to air current, transported uncovered, it would harbor many types of bacteria. FAO/WHO Experts committee (1962) and Wagner (2000).

In the Sudan, the meat is transported uncovered and also in butcher shops, meat is exposed to air current and house flies which are important sources of fecal contamination as it is displayed in open spaces. In addition to cutting and boning which are carried out by unclean contaminated tools.

In the current study, *Escherichia coli* was predominant in most samples, it indicates contamination and poor sanitation. This finding agreed with Alaboudi *et al.* (1989), Borah *et al.* (1992), Fatima (1990), and Fathi *et al.* (1992).

The result of this study revealed that, *Salmonella* spp were not detected in any sample examined although meat and meat products were most frequently associated with Salmonella (Hubbert *etal.*, 1975, Frazier and Westhoff, 1978, Wagner, 2000 and Cheesbrough, 2000). This result is in agreement with Hussein (1987) who did not detect Salmonella in his study.

The frequency bacteria detected were *Microccocus* and *Bacillus*. . This is in agreement with Result of Salih (1971) and Riemann (1969) showed that fresh meat samples were heavily contaminated with spoilage bacteria of the genera *Micrococcus* and *Bacillus*.

Our results substantiated such study in isolating *Aeromonas salmonicida* and *A.hydrophila*. Results of Egan (1984). Demonstrated a low incidence of *Aeromonads* in the investigated samples. These results were similar to those of Stern *et al*., (1987) who isolated these organisms from only 4 out of 99 fecal samples.

Also the result of Sanousi *et al.,* (1986) who isolated *A.hydrophila* from meat products agreed with results obtained in this study.

Among the Gram –positive bacteria that were found to contaminate meat samples were the *Kurthia gibsonii.* This finding agreed with Keddie (1981) such bacterium Was found on beef and mutton samples but not on camel meat.

The interactions of some groups of spoilage organisms can be usually found in refrigerated meat stored in air, such as *Enterobacteriaceae*, *Pseudomonas*, *Acinetobacter*, *Psychrobacter*, *Shewanella*, *Carnobacterium*, *Lactobacillus*, *Leuconostoc*, *Brochothrix* and *Kurthia spp*. The growth of these organisms was studied in the range

of temperature 2–11°C and pH 5.2–6.4, which is characteristic of refrigerated meat (Carmen and József, 1998). Butcher's shops refrigerator always contain *K.gibsonii* in refrigerated beef and mutton but not camel meat. This could be due to the fact that camel meat is brought from abattoirs and sold immediately without refrigerating.

In this study four species of Vibrio were detected or isolated from meats that's mean the pH of meats were alkaline and this indicates spoilage of meates ,because pH of meats is neutral.

Aeromonas spp are greatly affected by elevated temperatures above 45˚C (Hazen, 1979, Hazen and Fliermans, 1979, Riippey and Cabelli, 1979 and Silliker *et al.*, 1980). This is in agreement with this study in that the *Aeromonas hydrophila* and *A.salmonocida* were killed by the different temperatures applied in this study.

Nashwa (2004) boiled the pasteurelized milk to kill the isolated bacteria from milk viz: *Staphylococci*, *Micrococci*, *Streptococci*, *Stomatococci*, *Bacillus spp*, *Corynebacterium*, *Listeria ,Rothia spp, Pasteurella spp, Moraxella spp, Vibrio spp, Flavobacterium spp, Shigella spp*, *Achromobacter spp*, *Acinetbacter spp*, *Branhamella spp* also she determined the thermal death time of the isolated organisms by the boiling. The thermal death time ranged between 2-10 minutes and more. This agrees with results obtained in this study, but differs in *Bacillus mycoides* which survived the boiling for more than 10 minutes.

The thermoduric organisms are two types, spore –formers and non spore- formers, which have a greater resistance to temperature that destroy the ordinary bacteria (Henderson, 1971). This finding is

substantiated with this study in which *B.mycodies* was found to be resistant boiling for 10 minutes or more.

All organisms under test were killed by the different temperatures applied except *Bacillus mycoides*, which survived boiling for more than 10 minutes.

Gram-positive cocci are frequent organisms on meats in contrast to Gram-negative rods. *Escherichia coli, Micrococcus spp, V.parahaemolyticus ,Bacillus mycoides, kurthia gibsonii and Aeromonas salmonicida* were prevalent on the three types of meats. The occurance of *Kurthia spp* on meats indicates the refrigeration of meats.

GENERAL FOOD SAFETY TIPS

There are basic steps that should always be followed to help in reduction of the risk of foodborne illness:-

Clean: Wash hands, contact surfaces (e.g. kitchen counters) and utensils often to avoid the spread of bacteria.Wash hands with soap and warm water for at least 20 seconds before and after handling food, and after using the bathroom, changing diapers or touching pets.

Always wash fresh fruits and vegetables with clean, running water that is safe to drink.

Separate: Keep raw foods separate from cooked and ready-to-eat foods to avoid cross-contamination.

Ideally, use two cutting boards, one for raw meat, poultry and seafood, and one for washed fresh product and ready-to-eat foods.

Never place cooked food back on the same plate or cutting board that previously held raw food, unless it has been washed with soap and warm water.

Cook: Make sure you kill harmful bacteria by cooking foods to the proper internal temperature.

Use an instant-read digital thermometer and cook to these temperatures:

85°C (185°F) for whole poultry.

74°C (165°F) for stuffing, casseroles, leftovers, egg dishes, ground turkey and ground chicken, including sausages containing poultry meat

71°C (160°F) for pork chops, ribs and roasts, and for ground beef, ground pork and ground veal, including sausages.

At least 63°C (145°F) for all whole muscle beef and veal cuts, such as steaks and roasts

When you think the food is almost ready, remove it from the heat source and insert the thermometer in the thickest part of the food, away from bone, fat or gristle. Resume cooking if the proper temperature has not been reached.

Be sure to wash the thermometer in between temperature checks.

Eat hot foods while they are still hot.

Pathogenic bacteria cause illness. They grow rapidly in the "Danger Zone" – the temperatures between 40 and 140 °F – and do not generally affect the taste, smell, or appearance of food. Food that is left too long at unsafe temperatures could be dangerous to eat, but smell and look just fine. *E. coli* O157:H7, *Campylobacter*, and *Salmonella* are examples of such pathogenic bacteria.

Hygienic production of carcass meat is essential to ensure that contamination with potentially pathogenic bacteria is minimised. One must consider that such organisms will, at times, be present on product, albeit in low numbers. The potential for proliferation of food borne pathogens and spoilage organisms can be reduced by rapid chilling of product so as to limit bacterial growth and avoid conditions suitable for toxins production.

Recommendations

1 - Use clean water to wash the dishes and equipment used in cutting and Skinning.

2 –wash carcasses by clean water.

3 - The use of private vehicles is not armored clean air for the transfer and distribution of meat markets and butcher shops.

4 - Constant washing and non-accumulation of dirt in places abattoirs and sale of meat.

5 – Wash meat well before cooking.

6 - Good for cooking meat and the use of appropriate temperature to kill the microbes.

7 - Good conservation of the meat cooked.

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